

CLAIMS

1. A method for binding signal-emitting entities to target/probe molecule pairs within features of an array, the method comprising:

binding primer linker molecules to target/probe molecule pairs within features of the array to form nascent complexes bound to target/probe molecule pairs; and

repeatedly binding additional linker molecules that include one or more signal-emitting entities to the nascent complexes until molecular complexes containing a sufficient number of signal-emitting entities are obtained.

2. The method of claim 1 wherein the complexes contain a sufficient number of signal-emitting entities when a signal can be detected from a feature containing target/probe molecular pairs bound to the complexes.

3. The method of claim 1 wherein repeatedly binding additional linker molecules that include one or more signal-emitting entities to the nascent complexes further includes:

binding a first set of linker molecules including one or more signal-emitting entities to linker molecules previously bound to the complexes; and

binding a second set of linker molecules including one or more signal-emitting entities to linker molecules of the first set of linker molecules previously bound to the complexes.

4. Results, stored in a computer-readable medium, produced by detecting signals from an array to which signal-emitting entities are bound to target/probe pairs by the method of claim 1.

5. Results, transferred to an intercommunicating entity via electronic signals, produced by detecting signals from an array to which signal-emitting entities are bound to target/probe pairs by the method of claim 1.

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6. Results, printed in a human-readable format, produced by detecting signals from an array to which signal-emitting entities are bound to target/probe pairs by the method of claim 1.

7. A system for generating and amplifying signals from target/probe molecule pairs bound to an array, the system comprising:

an array having features containing target/probe molecule pairs;

a primer linker solution used to bind a primer linker to target/probe molecule pairs to generate a nascent molecular complex;

a solution containing a first set of linker molecules that include signal-emitting entities used to associate linker molecules of the first set of linker molecules to linker molecules previously incorporated in the complex;

a solution containing a second set of linker molecules that include signal-emitting entities used to associate linker molecules of the second set of linker molecules to linker molecules previously incorporated in the complex; and

a covalent-binding mediator that mediates covalent binding of associated linker molecules.

8. A method for binding signal-emitting entities to a complementary polynucleotide-target/polynucleotide-probe pair bound to an array, the method comprising:

generating a blunt end at an unbound terminus of the polynucleotide-target/polynucleotide-probe pair;

binding a primer oligonucleotide linker to the blunt end of the unbound terminus of the polynucleotide-target/polynucleotide-probe pair to form a complex; and

repeatedly binding oligonucleotide linkers including signal-emitting entities to oligonucleotide linkers previously bound to the complex.

9. The method of claim 8 wherein the complementary polynucleotide-target/polynucleotide-probe pair comprises an RNA target molecule hybridized to a probe DNA oligonucleotide.

10. The method of claim 9 wherein generating a blunt end at an unbound terminus of the polynucleotide-target/polynucleotide-probe pair further includes applying an arrayed primer extension technique to extend the probe molecule.

11. The method of claim 9 wherein generating a blunt end at an unbound terminus of the polynucleotide-target/polynucleotide-probe molecule pair further includes applying an exonuclease to digest unhybridized single-stranded regions of the target molecule.

12. The method of claim 9 wherein oligonucleotide linkers each comprises:

a first single-stranded DNA nucleotide polymer having first and second non-complementary regions; and

a second, anti-parallel DNA polynucleotide polymer having a first region that is base-sequence-complementary to the first region of the first DNA polynucleotide polymer and a second region non-complementary to either the first or second regions of the first DNA polynucleotide polymer and to the first region of the second DNA polynucleotide polymer, the base complementary regions of the first and second single-stranded DNA polynucleotides hybridizing to form a double-stranded body and the non-complementary regions of the first and second single-stranded DNA polynucleotides forming two single-stranded arms.

13. The method of claim 12 wherein non-primer oligonucleotide linkers each further comprises a third, single-stranded arm at an opposite end of the linker body from the two single-stranded arms.

14. The method of claim 13

wherein the primer oligonucleotide linker includes a first arm having a first base sequence and a second arm having a second base sequence; and

wherein non-primer oligonucleotide linkers include

a first set of non-primer oligonucleotide linkers that includes

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an oligonucleotide linker having a first arm with a third base sequence, a second arm with a fourth base sequence, and a third arm with a base sequence complementary to the first base sequence, and

an oligonucleotide linker having a first arm with a third base sequence, a second arm with a fourth base sequence, and a third arm with a base sequence complementary to the second base sequence; and

a second set of non-primer oligonucleotide linkers that includes

an oligonucleotide linker having a first arm with the first base sequence, a second arm with the second base sequence, and a third arm with a base sequence complementary to the third base sequence, and

an oligonucleotide linker having a first arm with the first base sequence, a second arm with the second base sequence, and a third arm with a base sequence complementary to the fourth base sequence.

15. The method of claim 14 wherein repeatedly binding oligonucleotide linkers including signal-emitting entities to oligonucleotide linkers previously bound to the complex further includes:

binding non-primer oligonucleotide linkers of the first set of oligonucleotide linkers to the molecular complex via hybridization of the third arms of the non-primer oligonucleotide linkers of the first set of oligonucleotide linkers to complementary arms extending from the complex; and

binding non-primer oligonucleotide linkers of the second set of oligonucleotide linkers to the molecular complex via hybridization of the third arms of the non-primer oligonucleotide linkers of the second set of oligonucleotide linkers to complementary arms extending from the complex.

16. A method for covalently binding layers of partially double-stranded oligonucleotide linkers onto a polynucleotide-target/polynucleotide-probe pair bound to an array to form a complex that can be detected by analysis, the method comprising:

covalently binding an initial partially double-stranded oligonucleotide linker to the polynucleotide-target/polynucleotide-probe pair, the initial partially double-stranded

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oligonucleotide linker having at least two single-stranded oligonucleotide arms and forming a complex with at least two single-stranded arms; and

repeatedly

covalently binding one or more next partially double-stranded oligonucleotide linkers to the complex following association of the single-stranded oligonucleotide arms of the complex to complementary single-stranded arms of the next partially double-stranded oligonucleotide linkers, each one or more next double-stranded oligonucleotide linker having a single-stranded oligonucleotide arm complementary to the to one or more of the single-stranded oligonucleotide arms of the complex and at least one single-stranded oligonucleotide arm not complementary to the single-stranded oligonucleotide arms of the complex and not complementary to the single-stranded oligonucleotide arms of the one or more next double-stranded oligonucleotide linkers.

17. The method of claim 16 wherein the initial partially double-stranded oligonucleotide linker has a blunt, double-stranded end and two single-stranded oligonucleotide arms at the end opposite from the blunt, double-stranded end.

18. The method of claim 16 wherein covalently binding an initial partially double-stranded oligonucleotide linker to the polynucleotide-target/polynucleotide-probe pair further comprises:

forming a blunt, free end on the polynucleotide-target/polynucleotide-probe pair; and

ligating the blunt end of the initial partially double-stranded oligonucleotide linker to the blunt, free end of the polynucleotide-target/polynucleotide-probe pair.

19. The method of claim 16 wherein each one or more next partially double-stranded oligonucleotide linker has a first end having one single-stranded oligonucleotide arm complementary to the to one or more of the single-stranded oligonucleotide arms of the complex, a double-stranded body, and a second end having two single-stranded oligonucleotide arms not complementary to the single-stranded oligonucleotide arms of the complex and not complementary to the single-stranded oligonucleotide arms of the one or more next double-stranded oligonucleotide linkers.

20. The method of claim 19 wherein repeatedly covalently binding one or more next partially double-stranded oligonucleotide linkers to the complex following association of the single-stranded oligonucleotide arms of the complex to complementary single-stranded arms of the next partially double-stranded oligonucleotide linkers further includes:

repeatedly

covalently binding a first set of partially double-stranded oligonucleotide linkers to the complex; and

covalently binding a second set of partially double-stranded oligonucleotide linkers to the complex.

21. The method of claim 19 wherein, after covalent binding of one or more next partially double-stranded oligonucleotide linkers to a complex with n single-stranded oligonucleotide arms, the resulting complex has approximately $2n$ single-stranded oligonucleotide arms.

22. Results, stored in a computer-readable medium, produced by analyzing an array containing complexes generated by the method of claim 16.

23. Results, transferred to an intercommunicating entity via electronic signals, produced by analyzing an array containing complexes generated by the method of claim 16.

24. Results, printed in a human-readable format, produced by analyzing an array containing complexes generated by the method of claim 16.

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